Optimization of CHO Transient Gene Expression (TGE) for Multi-Gram Level Antibody Production: Effects of Expression Construct, Post Transfection Cell Density and Feed Conditions

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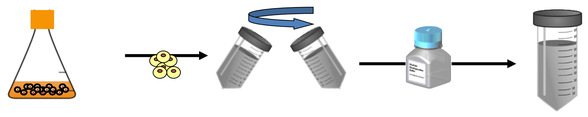
Abstract

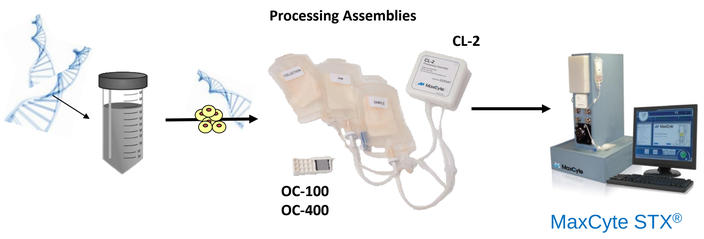
A variety of CHO cell transient transfection methods have been reported including systems based on engineered CHO cells, unique expression systems and specialized transfection reagents, but they each have varying levels of reproducibility, scalability, and cost effectiveness and generally produce antibody titers from 10 - 100mg/L (1-5). While these levels can be sufficient for very early studies, a transient CHO system that easily scales from milligram production to multi-gram quantities would be advantageous and enable the use of CHO transient gene expression (TGE) as a tool at even later stages within the biotherapeutic development pipeline. Flow electroporation technology from MaxCyte allows for highly reproducible, large scale TGE in CHO cells. Secreted antibody titers are routinely greater 500mg/L and can **exceed 1gram/L** with optimization. In this poster we present data for the development and fine tuning of several CHO-based antibody production systems that maximize antibody titers and laboratory productivity. The effects of multi- vs. tandem antibody expression plasmids, post transfection cell density, media and feeding conditions will be discussed. We demonstrate that CHO TGE using MaxCyte transient transfection can result in antibody titers >1.2 grams/L with the capacity to produce multi-gram quantities of antibodies from a single 30 minute electroporation.

Materials & Methods

Cell Harvesting



Electroporation



Cell Transfection Using the MaxCyte STX®: CHO cells were harvested and resuspended at 2E8 cells/mL in MaxCyte electroporation buffer. Cells were mixed with antibody construct(s) at 1-2µg/1E6 cells and transferred to single use processing assembly (PA). 400µl of cells (8E7 cells) were transfected via small scale transfection using static electroporation in OC-400 PAs; 15ml of cells (3.5E9 cells) were transfected via large scale transfections by flow electroporation in CL-2 PAs, unless otherwise noted. The MaxCyte STX comes pre-loaded with a library of electroporation protocols. The CHO-specific electroporation protocol was used.

Post Electroporation Culturing: After electroporation, cells were transferred to a sterile culture container and allowed to recover for 20 minutes at 37°C. Cells were suspended in medium (30ml for small scale transfections or 1.2L for large scale transfections) and cultured for up to 21 days. Post transfection cell densities at the time of seeding were 2E6, 6E6 or 1E7 cells/ml (see figure captions for details). For feed studies cells were suspended in one of two different media formulations and fed using two different schedules.

Antibody Production: Media samples were taken at various times post electroporation for antibody production analysis. Antibody titers were determined via ELISA or HPLC Protein A.

Increasing Antibody Titers by Optimizing Expression Constructs

Antibody Titers >700mg/L Over Sustained Culture Times

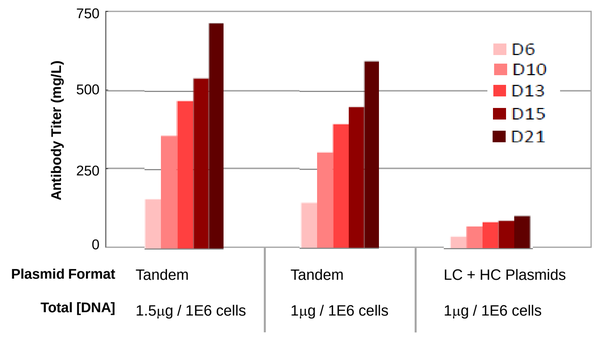


Figure 1. Antibody Titers Greater than 700 mg/L Using Optimized Expression Constructs. CHO cells were transfected with a single plasmid containing tandem heavy and light chain genes or co-transfected with two unoptimized plasmids expressing the heavy and light chain genes. Total DNA concentration was 1µg DNA/1E6 cells. Additionally, a set of CHO cells was transfected at a higher DNA concentration (1.5µg DNA/1E6 cells) with the tandem construct. Transfected cells were cultured at 2E6 cells/mL post electroporation. Antibody titers were determined using HPLC Protein A. Cells transfected with the tandem expression construct showed significantly higher antibody production with titers approaching 500mg/L at Day 13, with higher productivity for cells transfected with a higher DNA concentration. Because the cells retained high viability, cultures were maintained for an additional week (21 days total culture time post electroporation). Antibody titers increased throughout the 3 week period to greater than 700mg/L.

High Antibody Titers Maintained Upon Scale Up

Seamless Transfection Scale Up

Gram Level Production Within Two Weeks

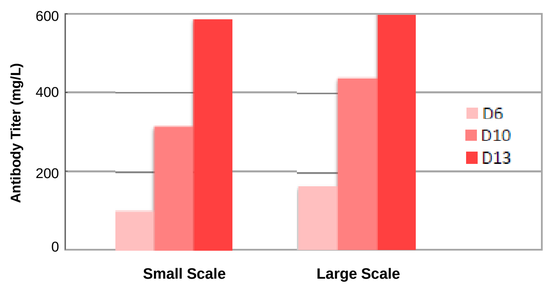


Figure 2. No Re-optimization of Methods Required for Transfection Scale Up. CHO cells were transfected with the tandem antibody expression plasmid (1.5µg DNA per 1E6 cells) using small or large scale electroporation, respectively. Cells were harvested using similar methods and the same electroporation parameters used for both transfections. Transfected cells were cultured at 2E6 cells/mL post electroporation. Total IgG concentrations were measured on days 6, 10 and13 post electroporation via HPLC Protein A. Cells transfected via small and large scale electroporation produced comparable antibody titers over the two week study period demonstrating the true scalability of MaxCyte electroporation. Overall, these data show that 3.5E9 CHO cells transfected using a single 30 minute electroporation run can yield greater than 1 gram of antibody within 2 weeks of transfection.

Productivity is Antibody Dependent

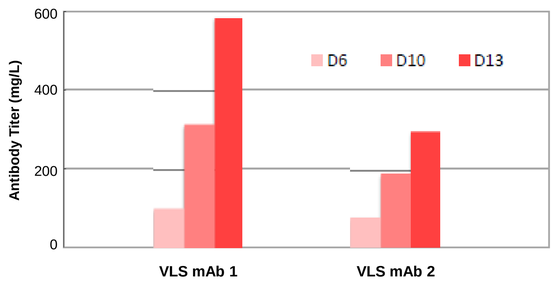


Figure 3. Antibody Specific Productivity. CHO cells were transfected with one of two tandem antibody expression plasmids (1.5µg DNA per 1E6 cells) using small scale electroporation. Transfected cells were cultured at 2E6 cells/mL post electroporation. Antibody titers were measured on days 6, 10 and13 post electroporation via HPLC Protein A. CHO cells produced significantly higher levels of VLS mAb1, with titers approaching 600mg/L on Day 13. CHO cells transfected with VLS mAb2 generated titers of approximately 250mg/L, which is well over typical yields from other CHO-based TGE systems (1-5).

Achieve 1.2 grams/L with Optimum Feeding

Media Composition and Feeding Schedule have Significant Impact on Antibody Production

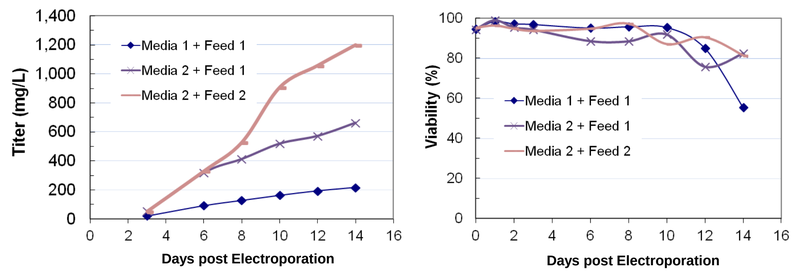


Figure 4. Gram Scale Antibody Titers. CHO cells were transfected using small scale electroporation. Post transfection, cells were suspended at approximately 4E6 cells/ml in either Media 1 or Media 2. Cells were fed using 2 different feeding schedules. The culture temperature was lowered to 32°C 24 hours post electroporation for all conditions. Total IgG concentrations were measured on days 3 -14 post electroporation using an ELISA. Both media composition and the feeding schedule impacted antibody production. By optimizing these post electroporation parameters we achieved an antibody titer of *1.2 grams/L*. These data were generated using small scale electroporation of 8E7 cells. The MaxCyte STX® can transfect up to 1E10 cells, while the MaxCyte VLX® can transfect up to 2E11 cells in a single 30 minute run. These scalable systems would easily provide a means of multi-gram antibody production in two weeks using transient transfection.

Increased Laboratory Productivity

Higher Cell Density Post Electroporation

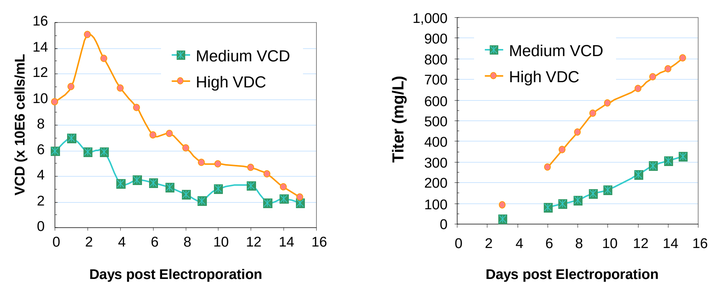


Figure 5. Increasing Post Electroporation Cell Density Increases Secreted Antibody Titers. CHO cells were harvested and resuspended at 2E8 cells/mL in 50% MaxCyte electroporation buffer. Cells were transfected with an antibody expression plasmid (1µg DNA per 1E6 cells) via large scale electroporation. Transfected cells were inoculated into two shake flasks at densities of 6 million or 10 million cells per mL, respectively. 1mM Na butyrate was added and the culture temperature lowered to 32°C 24 hours post electroporation. Cultures were fed daily. Cell density at the time of culture seeding impacted antibody expression and can be optimized for antibody yield or integration within manufacturing processes.

Summary

- The MaxCyte STX® Scalable Transfection System enables rapid transient transfection of CHO cells with high cell viability & transfection efficiency enabling CHO-based transient gene expression (TGE) for antibody production.
- Optimized post transfection conditions can generate antibody titers of **1.2 grams/L** and allows users to **generate multiple grams of antibody** from a single 30 minute transient transfection run.
- Antibody yield is dependent on expression construct, specific antibody, feeding parameters and post transfection cell density.
- High antibody titers are generated within two weeks of transfection. High cell viability allows culture times to be extended (total of 3 weeks post electroporation) further increasing antibody yields per transfection if desired.
- Optimization of the antibody expression constructs can improve antibody titers.
- MaxCyte's electroporation technology does not place restrictions on cell density allowing for optimization and streamlining of post transfection production methods.
- MaxCyte transient transfection produces consistent results at both small scale (static electroporation) and large scale (flow electroporation) without requiring any re-optimization.
- MaxCyte transient transfection of CHO cells does not require specialized expression constructs, engineered CHO cell lines or transfection reagents allowing users the flexibility to use their cell line, expression system and culture media of choice.

References

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